PB90-106311

Effect of Sediment Organic Carbon on Survival of 'Hyalella azteca' Exposed to DDT and Endrin

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1989

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1. REPORT NO. EPA/600/J-89/117	2.
3.	PB90-106311
4. TITLE AND SUBTITLE: Effect of sediment organic carbon on survival of *Hyalella azteca* exposed to DDT and endrin	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): A.V. Nebeker, G.S. Schuytema, W.L. Griffis, J.A. Barbitta, and L.A. Carey	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: USEPA, Corvallis, OR	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: US Environmental Protection Agency, Environmental Research Laboratory, 200 SW 35th Street, Corvallis, OR 97333	13. TYPE OF REPORT AND PERIOD COVERED: Journal Article
	14. SPONSORING AGENCY CODE: EPA/600/2

15. SUPPLEMENTARY NOTES

Environmental Toxicology and Chemistry, 8: 705-718, 1989

16. ABSTRACT

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Keywords: *Hyalella azteca*, DDT, Endrin, Sediment toxicity

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES: 16
	20. SECURITY CLASS (This page): Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

EPA/600/J-89/117

Environmental Toxicology and Chemistry, Vol. 8, pp. 705–718, 1989
Printed in the USA. Maxwell Pergamon Macmillan plc

0730-7268/89 $3.00 + .00

EFFECT OF SEDIMENT ORGANIC CARBON ON SURVIVAL OF *HYALELLA AZTECA* EXPOSED TO DDT AND ENDRIN

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(*Received* 8 *July* 1988; *Accepted* 19 *January* 1989)

Abstract—Sediment toxicity tests were conducted with the freshwater amphipod *Hyalella azteca* using sediments ranging in total organic carbon (TOC) concentration from 3 to 11% and spiked with either DDT or endrin. While the toxicity in the DDT-spiked sediment decreased with increasing TOC content, the TOC content of the endrin-spiked sediment had little apparent effect on toxicity. The 10-d sediment dry-weight LC50 for DDT was 11.0 μg/g at 3.0% TOC and 49.7 μg/g at 10.5% TOC. The sediment dry weight LC50s for endrin at 3.0 and 11.2% TOC were 4.4 and 6.0 μg/L, respectively. These results are important in evaluating the carbon normalization theory, which assumes that the availability of nonpolar organic compounds is reduced in the presence of higher concentrations of TOC. The differences in adsorption and toxicity between DDT and endrin are presumably related to the smaller carbon (K_{oc}) and octanol/water (K_{ow}) partition coefficients of endrin.

Keywords—*Hyalella azteca* DDT Endrin Sediment toxicity

INTRODUCTION

The organic carbon content of sediments is becoming recognized as a major factor in determining the bioavailability and toxicity of sediment contaminants to benthic biota. Sediments with higher concentrations of total organic carbon (TOC) have a greater capacity to adsorb nonpolar organic compounds, removing them from the interstitial water and from contact with the organisms and thereby reducing their toxicity [1,2]. Dissolved organic matter in sediment interstitial water can also reduce the bioavailability of organic pollutants [3,4]. Ziegenfuss et al. [1], evaluating the toxicity of kepone-spiked sediment to the midge *Chironomus tentans*, showed that as the concentrations of kepone and organic carbon in the sediment increased the toxicity of the pore water remained constant.

Little information is available on the toxicity of DDT or endrin to the amphipod *Hyalella azteca* (Saussure) (Talitridae), and there are no data for this benthic crustacean in the ambient water quality criteria documents for DDT [5] or for endrin [6]. Acute 96-h LC50 values for the amphipods *Gammarus fasciatus* and *Gammarus lacustris* in static tests have ranged from 1 to 9 μg/L for DDT [7–9] and from 3.0 to 5.5 μg/L for endrin [7–9]. *H. azteca* is widely distributed in the United States and is an important link in many aquatic food chains [10]. The ubiquity of this organism and the ease with which it can be cultured has made it a useful animal for experimental purposes [11,12]. *Hyalella* has been used in toxicity tests [13,14] and in sediment bioassays [12,15,16].

The present tests were conducted to help evaluate the potential use of established water quality criteria, corrected for organic carbon effects, in the formulation of sediment criteria. The study evaluated the organic carbon normalization theory [4] with respect to the bioavailability and toxicity of the nonpolar organic compounds DDT and endrin to the freshwater amphipod *Hyalella azteca*. The theory assumes that the acute toxicity of nonpolar organic compounds in the sediment is related to the TOC concentration in sediments and the concentration of toxic compounds within the pore water. Ten-day static tests to determine LC50 values were conducted using sediments with three different TOC levels. LC50 values in each test were calculated from DDT or endrin concentrations in sediment, uncentrifuged column water (total), centrifuged

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column water (soluble), interstitial or pore water, and water from test vessels run without sediment.

MATERIALS AND METHODS

Sediment

Two sources of uncontaminated sediment (verified by previous bioassays and chemical analyses) were used in the experiment. The first source, Soap Creek Pond No. 7, is a 0.2-ha pond located on Oregon State University land north of Corvallis, Oregon. The second, Mercer Lake, is a 145-ha body of water located 8 km north of Florence, Oregon. The Soap Creek Pond sediment was collected at a depth of 3 m with an Ekman grab sampler. Overlying water in the sampler was poured off during collection. The sediment was maintained for several months until needed in a 15-cm layer at the bottom of outdoor 75-liter aquaria supplied with flowing well water (10–17°C). The Mercer Lake sediment was collected with Ekman and Ponar grab samplers at a depth of 10 m. The sediment was placed in large polyethylene bags in ice chests and refrigerated at 4°C for up to 9 months until needed. The Soap Creek Pond sediment contained 3% TOC and the Mercer Lake sediment contained 11 to 12% TOC. A 1:1 mixture [4] of these two sediments was used as a third test sediment; it contained 6.1 to 7.2% TOC (measured values).

The sediment was spiked with five different concentrations of pesticide by being shaken in flasks partially coated with DDT or endrin. Each pesticide was dissolved in 10 ml ethyl acetate and was then coated on the inside of 2-liter narrow-mouth Erlenmeyer flasks with a rotary evaporator. Approximately 1.6 to 1.8 kg wet sediment was added to each flask and was shaken at 150 oscillations/min at room temperature for 7 d. This exposure time was chosen because of the slow rate at which the pesticides were adsorbed into the sediments (in previous tests, some chemical was still on the sides of the flasks after several weeks of shaking). The sediment was then transferred into wide-mouth Erlenmeyer flasks and kept on a shaker for up to 6 to 8 weeks until used, further ensuring that the pesticide was adsorbed to the sediment. Sediment chemical concentrations were established to bracket the pore water LC50 concentrations. The amount of DDT or endrin in the sediment necessary to obtain the desired series of concentrations in the water was developed from previous range-finding tests.

Water

The water was obtained from a well near the Willamette River at Corvallis, Oregon. It is characteristically soft, with low but variable levels of dissolved constituents. It was adjusted to a total hardness of 200 mg/L as $CaCO_3$ by the addition of $CaSO_4 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$ and $NaHCO_3$ in a modification of a standard method [17] in order to maintain uniform quality through the test period and to prevent a potential depression of pH evident in preliminary tests with unbuffered well water.

Test organisms

The *Hyalella* were cultured in flowing well water using 10-liter aquaria, with periodic exchanges of animals to ensure genetic variability and to prevent inbreeding, which may occur in small, isolated populations [16]. Pelleted DDT- and endrin-free (<0.4 μg/g) rabbit food, dry-blended to reduce particle size, was used as the main food source and substrate; it was fed as required to maintain the cultures. Care was taken to prevent overfeeding. The cultures, which can be maintained for extended periods of time, were kept at 20°C on a 16 h light:8 h dark cycle, with a light intensity of about 430 lux at bench top. Under these conditions, all life stages, from new young animals to coupled adults, were present in the cultures.

The animals used for testing were about 2 months old. Juveniles and young adults were isolated from older, larger adults by pouring the entire contents of the 10-liter culture aquaria through a 1-mm mesh standard sieve screen into another container (e.g., 25 × 40 cm white porcelain pan). The largest juveniles and young adults (4 mm long) that passed through the screen were collected by pipette for the experiment. Older reproductive *Hyalella* retained by the screen were placed back in the aquarium. Young, smaller animals that passed through the screen but were not used for tests were transferred to another clean aquarium, and excess food and wastes were discarded. Routine screening once a month was also used during this series of DDT and endrin tests to collect animals of known age (4 mm long) retained by the screen. Young animals passing through the screen became large enough the following month to be retained by the screen and used for testing. Young animals of known age exhibit excellent survival; animals of unknown age from mixed laboratory cultures often exhibit unreliable survival in tests because older, reproducing adults die. The *Hyalella* for testing were transferred into holding containers to observe for injured animals a few hours before they were added to the test beakers. Each test beaker received 40 mg powdered pelleted rabbit food on days 3 and 7 of the test, to allow normal behavior and growth

to continue in case the sediment became food-depleted. The delay in feeding until the third day of testing forced *Hyalella* to forage in the sediment, giving it greater contact with potential toxicants.

Test conditions

The test procedures developed for *Hyalella* have evolved from the procedures of Nebeker et al. [15, 16] and Swartz et al. [18]. Pesticide-spiked sediment (200 ml) and overlying water (800 ml) were placed in each test chamber (1,000-ml beakers). The beakers were gently aerated and the test organisms were added (20 *Hyalella*/beaker). The *Hyalella* were observed periodically during the test and the findings recorded. Mortality was determined at the end of the 10-d test to determine LC50 values for the test chemical. The contents of the beaker were poured on a 0.3-mm mesh standard sieve screen and washed with a fine spray; the animals were counted and removed with forceps. Dissolved oxygen concentration, pH, water temperature, test chemical concentration, hardness, conductivity and alkalinity were measured at specified intervals in selected test chambers. A range-finding test in which *Hyalella* were exposed to a series of widely spaced concentrations of the test chemical (0.1, 1.0, 10, 100, 500 mg/kg wet wt.) was followed by a definitive test in which toxicant-spiked sediment at five concentrations (2, 4, 8, 16, 32 mg/kg wet wt.) was placed in three replicate 1,000-ml beakers.

The definitive tests for this study, as outlined in the Battelle protocol [4], were conducted with three sediments of differing TOC levels for both DDT and endrin. Each test used three sets of 18 1,000-ml beakers. The first set contained pesticide-spiked sediment, water and animals. There were five sediment concentrations of pesticide plus a control, all in triplicate. This set of beakers was used only to determine *Hyalella* mortality at the end of 10 d.

The second set of 18 beakers was identical to the first and also contained spiked sediment, water and animals. Six beakers of this set were sacrificed on days 0, 5 and 10 for chemical analysis of pesticide and other chemical parameters. This method of sampling allowed the water and sediment in the first set of beakers to remain undisturbed by sampling until mortality was determined.

The third set of 18 beakers contained only spiked water and animals and was used to determine mortality in water-only systems. Samples for chemical analysis of the third set were collected from the stock of spiked water initially placed in the beakers on day 0, from pooled samples from replicate beakers on day 5, and from six of the beakers on day 10. Since beakers from the third set were not sacrificed as they were in the second set, it was necessary to pool some water on day 5 from each of the replicates to obtain sufficient sample for analysis. The water used in the water-only beakers was spiked with DDT or endrin in an acetone carrier. The control beakers in this set were spiked with the acetone carrier.

The sediment beakers in the DDT tests contained 200 ml spiked sediment and 800 ml water; the endrin beakers contained 175 ml spiked sediment and 825 ml water. The sediment was added to the bottom of the beakers. Water was then either poured carefully down the side of the beaker (DDT) or onto a removable plastic disc (endrin) to minimize disturbance of the sediment bed. The beakers were allowed to stand for 1 d before the animals were added. On day 0, aeration was started a few hours before addition of animals using a small-diameter glass-tube-tipped plastic air line (1 mm i.d.) submerged so the tip was halfway between the water and the sediment surface. Twenty *Hyalella* were then added to each beaker. All testing was conducted under the same light and temperature regimes used for the stock culture (16 h light, 20°C). The DDT tests were run in a temperature-controlled waterbath and the endrin tests were conducted in an environmental room.

Analytical procedures

Samples of water (100 ml) were extracted for DDT and endrin with ethyl acetate by liquid–liquid extraction with a magnetic stirrer [19,20]. The extracts were concentrated using a Kuderna–Danish concentrator [21]. Samples were then analyzed using fused-silica capillary column GC with electron capture detection on a Tracor 570 or Hewlett-Packard 5730 gas chromatograph operated in the split mode at a split ratio of 50 to 75:1. Sediment samples were analyzed by extracting with 1:1 ethyl acetate:acetone (v/v) in a serum bottle using the above method. Animal tissue was frozen after collection and extracted in a Potter–Elvehjem tissue grinder [22] with ethyl acetate. The extracts were brought to volume and analyzed by GC as above. Five or six standards were analyzed at the beginning of an analytical run, with a single standard analyzed after approximately every six samples. The U.S. Environmental Protection Agency (EPA) AQC sample WP385 was analyzed concurrently for *p,p'*-DDT. Recovery was 95% of the standard's reported value, with a coefficient of variation of 2% ($n = 6$).

The detection limits for DDT in water and sediment were 0.1 μg/L and 0.2 μg/g, respectively. The specific recovery of DDT in water, based on extraction and injection, was 99.6 ± 5.4% (mean ± SD, $N = 32$). The mean coefficients of variation for water and sediment sample replicate pairs were 6.5% ($N = 22$ pairs) and 13.8% ($N = 21$ pairs), respectively. The detection limits for endrin in water and sediment were 0.1 μg/L and 0.1 μg/g, respectively. The specific recovery of endrin in the test water, based on extraction and injection, was 103.2 ± 10% (mean ± SD, $N = 29$). The mean coefficients of variation for water and sediment sample replicate pairs were 4.1% ($N = 30$ pairs) and 6.6% ($N = 35$ pairs), respectively. The specific recovery of endrin in the test sediment was 101 ± 7.8% (mean ± SD, $N = 4$). The detection limit for endrin in tissue was 5 μg/g.

Water quality was characterized by chemical analysis prior to and during testing. Dissolved oxygen in the endrin tests was measured daily by electrode, and on days 0, 5 and 10 in the DDT tests by the Winkler titration method [23], when the beakers were sacrificed. Total hardness (EDTA titration in DDT tests), alkalinity, pH and conductivity were determined on days 0, 5 and 10 using EPA methods [23]. Hardness was calculated in the endrin tests from calcium and magnesium concentrations measured with an inductively coupled plasma-atomic emission spectrometer [24]. Total carbon in the sediment was determined by combustion and measurement of carbon dioxide with a Coulometrics carbon dioxide coulometer [25]. Total sediment inorganic carbon was determined by a modification of the calcimeter method of Allison and Moody [26]. TOC was the difference between the total and inorganic carbon values. Total and soluble organic carbon in the water was determined by coulometric detection [27]. Dry solids content of the sediment was determined using EPA methods [23].

Sediment was collected from sacrificed beakers for analysis by carefully decanting the overlying water, stirring the sediment and pouring it into centrifuge tubes or sample bottles. Samples for soluble DDT and endrin and for DDT and endrin in the pore water were centrifuged in 50-ml stainless steel tubes for 30 min. The DDT samples were spun at 17,500 rpm (37,000 *g*) and the endrin samples at 16,000 rpm (30,900 *g*). The stainless steel tubes and all glassware were washed with detergent, rinsed in tap and reverse-osmosis water, and either rinsed with acetone or baked overnight at 250°C before use.

Calculations

Mortality data from the three replicates for each concentration were pooled prior to calculation of the LC50s and 95% C.I. by the trimmed Spearman-Karber method [28]. Mean values for column water, pore water and sediment DDT and endrin concentrations were obtained by averaging the values observed on days 0, 5 and 10 of the tests. Dry weight DDT and endrin concentrations were derived by dividing the wet weight concentrations by the decimal value of percent dry solids. Dry weight LC50s were normalized for carbon content by dividing the LC50 values by the decimal value of percent organic carbon.

RESULTS

Water quality and mortality

Water quality conditions were sufficient to maintain a healthy environment for the test animals. Dissolved oxygen and pH in the endrin test beakers with and without sediment ranged from 7.8 to 9.1 mg/L and from 7.3 to 8.5, respectively. Total hardness, alkalinity and conductivity were lower in beakers containing sediment than in the water-only beakers, ranging from 93 to 199 mg/L, from 40 to 125 mg/L and from 400 to 750 μS, respectively. In the water-only beakers, the values for hardness were 178 to 199 mg/L, for alkalinity, 136 to 142 mg/L, and for conductivity, 560 to 700 μS.

Mean TOC water concentrations in beakers with endrin-spiked sediment ranged from 4.2 mg/L in the Soap Creek Pond tests to 8.8 mg/L in the Mercer Lake beakers. Mean soluble organic carbon water concentrations in beakers containing Soap Creek Pond sediment, 1:1 sediment mixture and Mercer Lake sediment spiked with endrin varied little between sites and were 2.0, 1.8 and 1.6 mg/L, respectively.

The mortality of control animals in the DDT sediment and water tests ranged from 0 to 5%. Control mortality in the endrin tests ranged from 6.7 to 8.3% with sediment and from 3.3 to 8.3% in tests with water only.

DDT

Pore water concentrations of soluble DDT (Table 1) did not increase from days 0 to 10, indicating that the pore water and sediment concentrations may have been in equilibrium at the start of the experiment. Mean concentration of total DDT in the water-only beakers ranged from 0.1 to 2.0 μg/L (Table 2). DDT toxicity based on both wet and dry sediment LC50s decreased with increasing

Table 1. Effect of three total organic carbon (TOC) levels in sediment on the toxicity of DDT to *Hyalella azteca*

Sediment tests[a]	% TOC in sediment	μg DDT[b] added/g wet sediment	Initial measured sediment DDT concn. (μg/g)	$\bar{x}$ (±SD) measured DDT in sediment (μg/g)		$\bar{x}$ (±SD) measured DDT in water (μg/L)			No. of *Hyalella azteca* dead[d] ($n = 60$)
				Wet wt.	Dry wt.[c]	Total	Soluble	Pore	
Soap Creek Pond No. 7	3.0	43	15.0	15.5 (1.6)	68.0 (7.0)	0.9 (0.6)	0.1 (0.0)	1.9 (1.7)	60
		21	11.0	11.3 (0.8)	49.6 (3.5)	1.3 (1.1)	<0.1	3.4 (3.2)	60
		10	4.0	4.8 (0.7)	21.1 (3.1)	0.8 (0.4)	<0.1	2.1 (1.2)	52
		5	1.0	1.2 (0.3)	5.3 (1.3)	0.2 (1.6)	<0.1	0.3 (0.2)	4
		2	0.4	0.5 (0.1)	2.2 (0.4)	0.1 (0.0)	<0.1	<0.1	3
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	0
1:1 mixture (Soap Creek Pond and Mercer Lake)	7.2	85	72.0	71.9 (13.5)	415 (78)	6.1 (3.2)	0.8 (0.1)	–	60
		42	26.0	24.3 (1.5)	140 (8.7)	2.7 (1.6)	0.5 (0.1)	8.0 (2.8)	60
		21	17.0	15.3 (1.6)	88.4 (9.2)	2.4 (2.7)	0.3 (0.1)	4.4 (1.7)	60
		11	5.0	5.3 (1.1)	30.6 (6.4)	1.8 (1.3)	0.2 (0.1)	2.0 (0.6)	39
		4	1.0	1.0 (0.0)	5.8 (0.0)	0.5 (0.4)	0.1 (0.1)	0.7 (0.4)	6
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	3
Mercer Lake	10.5	42	28.0	29.5 (3.5)	198 (24)	3.8 (2.2)	0.4 (0.0)	5.5 (0.4)	60
		21	18.0	19.7 (10.6)	132 (71)	3.0 (1.9)	0.1	1.6 (0.5)	58
		10	12.0	11.8 (0.3)	79.2 (2.0)	1.3 (0.8)	0.1	1.3 (0.0)	43
		6	6.0	6.9 (1.0)	46.3 (2.0)	0.8 (0.3)	<0.1	1.1 (0.1)	29
		3	3.0	3.3 (0.3)	22.1 (2.0)	0.1	<0.1	0.2 (0.2)	3
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	0

[a]Percent dry solids: Soap Creek Pond, 22.8%; 1:1 mixture, 17.3%; Mercer Lake, 14.9%.
[b]Coated on sides of flasks in which wet sediment was spiked; not all DDT was adsorbed by the sediment.
[c]Dry weight DDT is derived, not measured, value (wet weight/% dry solids).
[d]Three beakers, 20 animals/beaker.

Table 2. Survival of *Hyalella azteca* in water-only DDT tests

Water-only test[a]	Nominal water DDT concn. (μg/L)	$\bar{x}$ (±SD) measured total DDT (μg/L)	No. of *Hyalella azteca* dead[b] (n = 60)
Run with Soap Creek Pond No. 7 sediment test	4.1	2.0 (2.3)	60
	2.4	1.5 (1.8)	59
	1.0	0.6 (0.5)	35
	0.5	0.2 (0.2)	10
	0.2	0.1 (0.0)	3
	0.0	<0.1	1
Run with 1:1 mixture (Soap Creek Pond and Mercer Lake) sediment test	4.1	1.3 (1.3)	60
	2.4	0.3 (0.3)	21
	1.0	0.5 (0.4)	20
	0.5	0.2 (0.2)	3
	0.2	0.1 (0.0)	4
	0.0	<0.1	3
Run with Mercer Lake sediment test	4.1	1.8 (2.1)	60
	2.4	1.0 (1.1)	45
	1.0	0.4 (0.3)	21
	0.5	0.2 (0.2)	9
	0.2	0.1 (0.1)	0
	0.0	<0.1	0

[a]Run concurrently with sediment tests as control tests.
[b]Three beakers, 20 animals/beaker.

Table 3. 10-d LC50 values for DDT in sediment on wet and dry weight basis

Sediment	% total organic carbon[a]	% dry[a] solids	DDT LC50 values (95% C.I.) Wet sediment (μg/g)	Dry sediment (μg/g)	Total organic carbon[b] (μg/g)
Soap Creek Pond No. 7	3.0	22.8	2.5 (2.3–2.9)	11.0 (10.1–12.7)	366.7 (336.7–423.3)
1:1 mixture (Soap Creek Pond and Mercer Lake)	7.2	17.3	3.4 (2.7–4.2)	19.6 (15.6–24.3)	272.2 (216.6–337.5)
Mercer Lake	10.5	14.9	7.4 (6.6–8.4)	49.7 (44.2–56.3)	473.3 (421.0–536.2)

[a]Measured values.
[b]Dry sediment LC50s normalized for carbon by dividing each DDT LC50 concentration by the decimal value of total organic carbon.

sediment organic carbon (Table 3, Fig. 1). LC50 values based on sediment wet weight increased from 2.5 μg/g at 3.0% TOC to 7.4 μg/g at 10.5% TOC. Dry weight LC50s followed the same pattern, increasing from 11.0 to 49.7 μg/g. When the dry weight LC50s were normalized for sediment organic carbon content, the results were variable, with the highest and lowest toxicities occurring at sediment TOC levels of 7.2 and 10.5%, respectively. LC50 values for DDT in water with sediment indicated the lowest toxicity in sediment containing 7.2% TOC (Table 4, Fig. 2). Pore water LC50s followed the same pattern and were similar to the total DDT values. LC50s for the water-only systems for each test were very similar and ranged from 0.46 to 0.52 μg/L (Table 4).

Endrin

Mean measured pore water concentrations of endrin (Table 5) in the Soap Creek Pond and Mercer Lake sediment tests did not increase over time, indicating that equilibrium may have been attained at the start of the experiment. Mean endrin pore water concentrations in the 1:1 mixture sediment

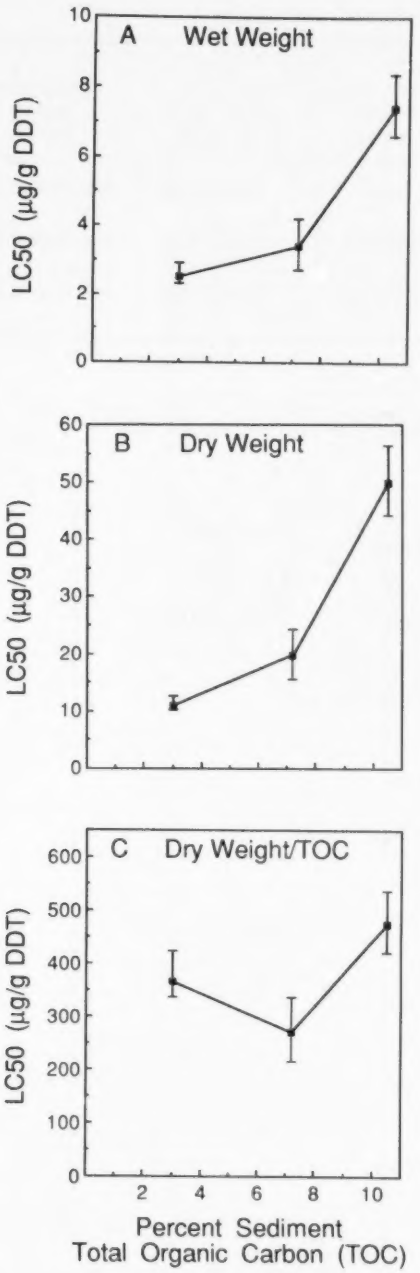

Fig. 1. LC50 values (95% C.I.) for DDT at three total organic carbon (TOC) levels in sediment on wet and dry weight basis. **A**: Wet sediment. **B**: Dry sediment. **C**: TOC-normalized (divided by percent TOC) dry sediment.

showed a small increase over time. Concentrations of mean total and mean soluble endrin in the water-only beakers ranged from 0.2 to 13.2 and from 0.3 to 12.0 µg/L, respectively (Table 6). There was little difference in endrin toxicity with increasing sediment organic carbon content (Table 7, Fig. 3). LC50 values ranged from 0.9 to 1.0 µg/g on a wet weight basis and from 4.4 to 6.0 µg/g on a dry weight basis. LC50 values normalized for sediment organic carbon indicated increasing toxicity with increasing sediment TOC levels. Error bars indicate little difference between the LC50 values at 6.1 and 11.2% sediment TOC. LC50 values for endrin in the water with sediment indicated little difference in toxicity for either total, soluble or pore water endrin (Table 8, Fig. 4). The water-only system LC50s were also very similar and ranged from 4.2 to 4.8 µg/L for total endrin and from 3.8 to 4.3 µg/L for soluble endrin.

DISCUSSION AND CONCLUSIONS

Hyalella was used in this series of tests as a representative macroinvertebrate, with younger animals critical for maintaining good survival in control and test containers [16]. *Hyalella* remain in intimate contact with the sediments when feeding and hiding, possibly as much as sediment animals that make burrows, but they have little more contact than do "surface" sediment feeders. The static 10-d procedures approximate worst case conditions, as potential toxic materials will be retained in the water in the beakers or those bound to particulates may be available to the animals when they feed or come into contact with the sediment. The 10-d time period of the test is rather arbitrary but is widely used [18]; it bridges the gap between the classic 96-h acute test and longer chronic tests. A test time longer than 10 d may cause undesirable changes in the test chambers, such as physicochemical modification of the test substance, adsorption of the chemical by uneaten food, bacterial and fungal growth, and stress. Cost is a significant factor and may be a consideration instrumental in selecting a shorter time period, such as 96 h. In most cases in which sediment tests are used, the additional set of beakers for chemical analysis only will not be needed; they were integral to this particular study and are not recommended for routine sediment testing with *Hyalella*.

At the start of the DDT exposures, after the sediment and water had been in contact for 24 h, it appeared that equilibrium had been established. The possibility exists that the system was in a steady

Table 4. 10-d LC50 values (95% C.I.) for DDT in water

Sediment	Water column[a]		Pore water[b] (μg/L)	Water-only total[c] (μg/L)
	Total (μg/L)	Soluble[b] (μg/L)		
Soap Creek Pond No. 7 (3.0% TOC)	0.43 (0.4–0.5)	<0.1	0.9 (0.8–1.0)	0.46 (0.39–0.55)
1:1 mixture (Soap Creek Pond and Mercer Lake) (7.2% TOC)	1.2 (1.1–1.4)	0.16 (0.15–0.18)	1.5 (1.3–1.8)	0.48 (0.44–0.52)
Mercer Lake (10.5% TOC)	0.6 (0.5–0.8)	0.10 (0.1–0.1)	0.8 (0.7–0.9)	0.52 (0.45–0.60)

TOC, total organic carbon.
[a]Water over sediment.
[b]Centrifuged at 17,500 rpm for 30 min.
[c]Non-renewal tests conducted with water only, done concurrently with water and sediment tests.

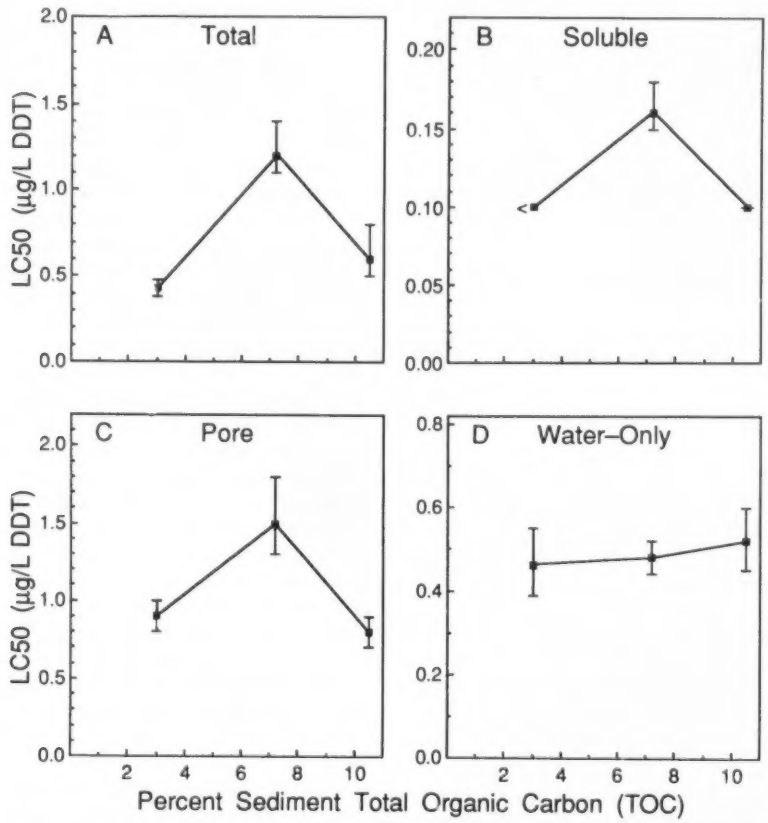

Fig. 2. LC50 values (95% C.I.) for DDT in water. **A**: Total DDT in water column over sediment. **B**: Soluble (centrifuged) DDT in water over sediment. **C**: DDT in pore water centrifuged from sediment. **D**: Total DDT in water-only tests without sediment.

Table 5. Effect of three total organic carbon (TOC) levels in sediment on the toxicity of endrin to *Hyalella azteca*

Sediment tests[a]	% TOC in sediment	μg endrin[b] added/g wet sediment	Initial measured sediment endrin concn. (μg/g)	$\bar{x}$ (±SD) measured endrin in sediment (μg/g)		$\bar{x}$ (±SD) measured endrin in water (μg/L)			No. of *Hyalella azteca* dead[d] (n = 60)
				Wet wt.	Dry wt.[c]	Total	Soluble	Pore	
Soap Creek Pond No. 7	3.0	25	12.0	10.5 (0.4)	45.9 (1.8)	16.3 (6.7)	15.2 (6.1)	23.8 (1.6)	60
		11	4.0	4.1 (0.2)	17.9 (0.1)	6.2 (2.6)	5.8 (2.3)	9.8 (0.6)	60
		6	2.0	1.8 (0.3)	8.1 (1.7)	3.0 (1.7)	2.6 (1.2)	4.7 (0.6)	54
		3	1.0	0.8 (0.1)	3.4 (0.5)	1.4 (0.5)	1.0 (0.4)	1.5 (0.3)	19
		2	0.5	0.5 (0.0)	2.2 (0.0)	0.9 (0.6)	0.7 (0.3)	1.1 (0.1)	12
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	4
1:1 mixture (Soap Creek Pond and Mercer Lake)	6.1	22	12.0	10.7 (1.2)	56.4 (6.1)	14.1 (5.1)	12.9 (2.6)	24.5 (5.4)	60
		13	6.0	6.0 (0.0)	31.7 (0.0)	7.5 (3.2)	6.4 (2.5)	10.6 (2.0)	60
		2	3.0	3.3 (0.6)	17.7 (3.1)	4.5 (2.1)	3.5 (1.4)	6.8 (1.3)	57
		3	0.5	0.9 (0.1)	4.9 (0.6)	1.7 (1.0)	1.0 (0.5)	1.7 (0.2)	26
		2	0.3	0.2 (0.0)	1.1 (0.2)	0.4 (0.2)	0.3 (0.2)	0.5 (0.3)	5
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	5
Mercer Lake	11.2	24	14.0	11.0 (1.1)	73.8 (6.7)	11.6 (4.6)	11.7 (4.9)	15.6 (1.0)	60
		13	5.0	4.0 (0.0)	26.8 (0.0)	4.2 (1.9)	4.0 (1.5)	7.2 (1.3)	60
		6	2.0	1.0 (0.0)	6.7 (0.0)	2.4 (2.1)	1.4 (0.7)	2.3 (0.2)	31
		3	1.0	0.2 (0.1)	1.1 (0.8)	1.1 (1.5)	0.3 (0.1)	0.3 (0.2)	1
		1	0.5	0.2 (0.1)	1.3 (0.0)	0.3 (0.2)	0.3 (0.2)	0.3 (0.1)	3
		0	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	4

[a]Percent dry solids: Soap Creek Pond, 22.8%; 1:1 mixture, 18.9%; Mercer Lake, 14.9%.
[b]Coated on sides of flask in which wet sediment was spiked; not all endrin was adsorbed by the sediment.
[c]Dry weight endrin is derived, not measured, value (wet weight/% dry solids).
[d]Three beakers, 20 animals/beaker.

Table 6. Survival of *Hyalella azteca* in water-only endrin tests

Water-only test[a]	Nominal water endrin concn. (μg/L)	$\bar{x}$ (±SD) measured endrin in water (μg/L)		No. of *Hyalella azteca* dead[b] ($n = 60$)
		Total	Soluble	
Run with Soap Creek Pond No. 7 sediment test	9.9	7.5 (1.8)	6.1 (1.6)	51
	3.7	2.9 (0.8)	2.8 (1.0)	6
	1.7	1.4 (0.4)	1.3 (0.5)	5
	0.6	0.5 (0.1)	0.8 (0.1)	3
	0.2	0.2 (0.1)	0.3 (0.1)	3
	0.0	<0.1	<0.1	3
Run with 1.1 mixture (Soap Creek Pond and Mercer Lake) sediment test	16.1	13.2 (2.3)	12.0 (2.3)	60
	7.5	6.6 (2.1)	5.1 (1.3)	42
	3.7	2.7 (0.9)	2.6 (1.2)	16
	2.4	2.2 (0.5)	1.8 (0.6)	9
	1.2	1.0 (0.2)	1.0 (0.3)	8
	0.0	<0.1	<0.1	5
Run with Mercer Lake sediment test	16.1	11.5 (4.0)	11.2 (3.4)	60
	7.5	5.1 (1.4)	5.4 (1.7)	39
	3.7	2.6 (0.8)	2.5 (1.0)	10
	2.4	1.8 (0.6)	1.7 (0.6)	5
	1.2	0.8 (0.3)	0.9 (0.2)	7
	0.0	<0.1	<0.1	2

[a]Run concurrently with sediment tests as control tests.
[b]Three beakers, 20 animals/beaker.

Table 7. 10-d LC50 values for endrin in sediment on wet and dry weight basis

Sediment	% total organic carbon[a]	% dry solids[a]	Endrin LC50 values (95% C.I.)		
			Wet sediment (μg/g)	Dry sediment (μg/g)	Total organic carbon (μg/g)
Soap Creek Pond No. 7	3.0	22.8	1.0 (0.9–1.2)	4.4 (3.9–5.2)	146.7 (130.0–173.3)
1.1 mixture (Soap Creek Pond and Mercer Lake)	6.1	18.9	0.9 (0.7–1.2)	4.8 (3.7–6.3)	78.7 (60.6–103.3)
Mercer Lake	11.2	14.9	0.9 (0.7–1.1)	6.0 (4.7–7.4)	53.6 (42.0–66.1)

[a]Measured values.
[b]Dry sediment LC50s normalized for carbon by dividing each endrin LC50 concentration by the decimal value of total organic carbon.

state between excess DDT spiked into the sediment and DDT in the pore water. As a result, the pore water may have been more representative of the concentration spiked into the sediment than of an equilibrium established between the sediment and pore water. However, by swirling the sediment in the flask with DDT adhering to the glass, it is still presumed that DDT was being adsorbed to the sediment.

Sediment DDT toxicity (μg DDT/g sediment) decreased with increasing TOC on both a wet and dry weight basis (Table 3, Fig. 1). There was no significant difference in toxicity of pore water between the lowest and highest TOC values; however, the toxicity of the intermediate TOC level was significantly less. We have no explanation for this deviation. Water column chemistry analyses revealed no differences that could have resulted in differ-

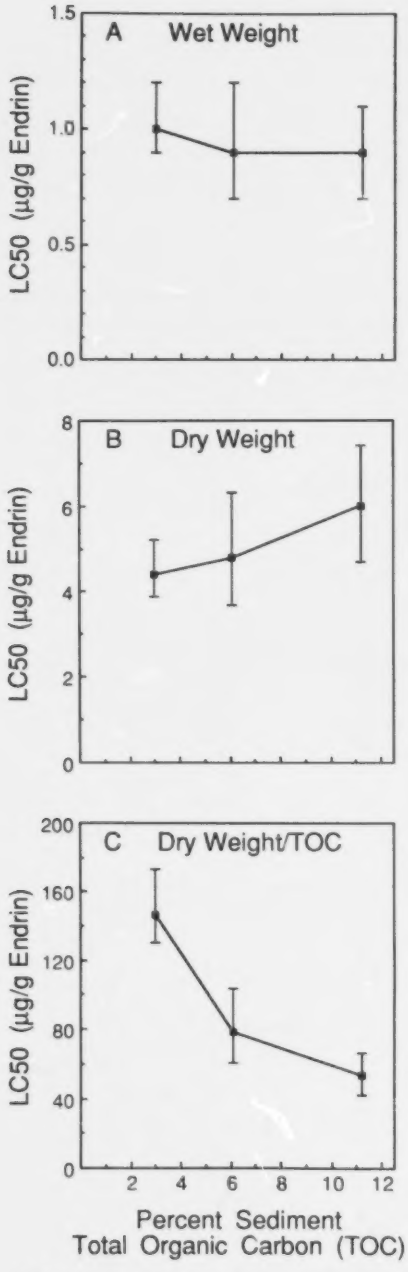

Fig. 3. LC50 values (95% C.I.) for endrin at three total organic carbon (TOC) levels in sediment on wet and dry weight basis. **A**: Wet sediment. **B**: Dry sediment. **C**: TOC-normalized (divided by percent TOC) dry sediment.

ences in complexation and subsequent lowering of toxicity (Table 4, Fig. 2). Ziegenfuss et al. [1] also noted a decrease in sediment toxicity with increasing sediment organic carbon, but showed little difference in pore water LC50s with increasing sediment organic carbon in tests with kepone. When our sediment DDT LC50s were normalized for TOC, the values were expected to be equal; considering the 95% C.I. ranges, the values obtained were fairly similar for the 3.0 and 10.5% levels of sediment TOC (Table 3, Fig. 1). The intermediate organic carbon sediment exposure was significantly lower than the highest TOC value, but it is not known what caused this nonlinear response to TOC normalization. Normalization of chemical concentration in the sediment to organic carbon was used by Adams et al. [29] to compare activity at different sites.

Sediment endrin LC50s indicated no difference in sediment toxicity due to TOC (Table 7, Fig. 3). When the LC50 values were normalized for TOC, there appeared to be an increase in toxicity with increasing TOC, in opposition to the normalization theory. In contrast, endrin pore water LC50 values (Table 8, Fig. 4) showed no significant difference in toxicity due to sediment TOC content.

The DDT results indicated that much of the DDT bound to the higher organic content sediment was not available to kill the organisms. The DDT in the water was most likely the primary cause of toxicity, since the pore water LC50s were independent of sediment DDT concentration. Unlike the DDT results, the endrin sediment LC50s indicated no difference in toxicity at higher sediment organic carbon levels. Ziegenfuss et al. [3] found the pore water in a kepone-spiked sediment system and the kepone-spiked water in a sediment-free system to be about equally toxic to the midge *Chironomus tentans*. In the present study, however, pore water endrin was more toxic than the water-only endrin, and pore water DDT was less toxic than water-only DDT.

In conclusion, the DDT data seemed to support the carbon normalization theory. That is, sediment toxicity decreased and pore water toxicity changed little with increasing sediment organic carbon. It is clear that the endrin data in the present study do not support equilibrium partitioning; neither pore water nor sediment LC50s indicated a decrease in toxicity with increasing sediment organic carbon. Physical differences between these two chemicals could account for the observed differences in toxicity and carbon relationships [30]. The partition

Table 8. 10-d LC50 values (95% C.I.) for endrin in water

Sediment	Water column[a]		Pore water[c] (μg/L)	Water-only[b]	
	Total (μg/L)	Soluble[c] (μg/L)		Total (μg/L)	Soluble[c] (μg/L)
Soap Creek Pond No. 7 (3.0% TOC)	1.7 (1.5–2.0)	1.3 (1.2–1.5)	2.1 (1.8–2.5)	4.8 (4.5–5.2)	4.2 (4.0–4.5)
1:1 mixture (Soap Creek Pond and Mercer Lake) (6.1% TOC)	1.6 (1.3–2.1)	1.1 (0.9–1.3)	1.9 (1.6–2.4)	4.4 (3.8–5.1)	3.8 (3.2–4.4)
Mercer Lake (11.2% TOC)	2.2 (2.0–2.5)	1.2 (1.0–1.4)	1.8 (1.4–2.2)	4.2 (3.8–4.8)	4.3 (3.8–4.9)

[a]Water over sediment.
[b]Non-renewal tests conducted with water only, done concurrently with water and sediment tests.
[c]Centrifuged at 16,000 rpm for 30 min.

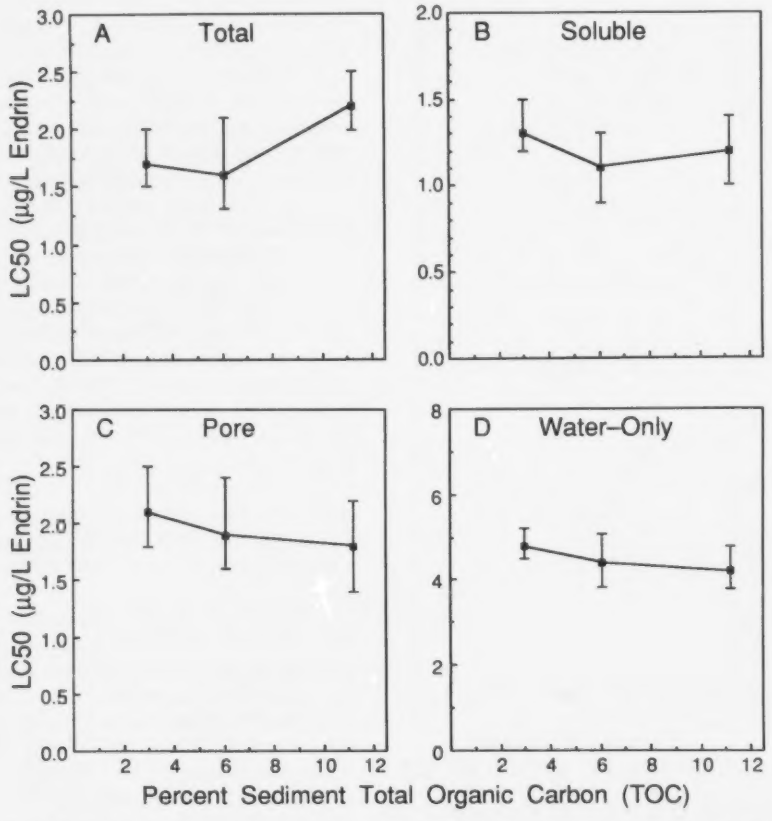

Fig. 4. LC50 values (95% C.I.) for endrin in water. **A**: Total endrin in water column over sediment. **B**: Soluble (centrifuged) endrin in water over sediment. **C**: Endrin in pore water centrifuged from sediment. **D**: Total endrin in water-only tests without sediment.

coefficient based on carbon content (K_{oc}) for DDT is 4×10^6, while that of endrin is only 1.7×10^3. Similarly, the octanol/water partition coefficients (K_{ow}) for these two chemicals are 8×10^6 and 3.5×10^3, respectively. It appears that the adsorptive capacity of sediment carbon is much less for endrin than for DDT. Differences in K_{oc} will result in a different amount of chemical partitioning into the pore water (endrin > DDT). However, if the carbon normalization theory holds, normalizing to organic carbon should work with either high or low K_{oc}. Normalizing the endrin data to TOC did not normalize the toxicity of the endrin across organic carbon treatments; carbon normalization did not hold for endrin in the present study. In fact, endrin was actually more toxic at high TOC when data were normalized for percent organic carbon (Fig. 3).

These results bear on the application of carbon-corrected water quality criteria to pore water in the evaluation of sediment criteria and in assessing the environmental hazard of sediments contaminated with organic compounds. The assumption that increased levels of sediment organic carbon will mediate the effects of organic toxicants obviously depends on the particular properties of the contaminant, especially with regard to carbon partitioning.

Acknowledgement—This study was part of a contract from the Environmental Protection Agency, Criteria and Standards Division (C. Zarba, Project Officer) in cooperation with Battelle Northwest Laboratories. We thank Spencer Peterson, Daniel Krawczyk, Clifford Miller and Merline Robideaux for advice and technical assistance, and Nancy Lanpheare for manuscript preparation.

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